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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of: OZANICH	Date: October 3, 2003
Serial No. 09/804,613	Group Art Unit: 2877
Filed: March 12, 2001	Examiner: F. L. Evans
For: AN APPARATUS AND METHOD FOR MEASURING AND CORRELATING CHARACTERISTICS OF FRUIT WITH VISIBLE/NEAR INFRA-RED SPECTRUM	

AMENDMENT AND RESPONSE

Hon. Commissioner of Patents and Trademarks
Washington, D.C. 20231

Dear Commissioner:

In response to the communication from the Examiner dated June 4, 2003, please consider the following:

Petition for Extension for Response Within the First Month-Fees Due

The applicant respectfully observes that this response is filed within the first month. The Examiner is hereby authorized to deduct $55.00 as fees for filing within the first month and to deduct other fees owing from the deposit account of Liebler, Ivey & Connor, P.S./Floyd E. Ivey, 35,552, Deposit account No. 50-0607.

Certificate of facsimile filing on October 3, 2003 by Floyd E. Ivey. Floyd E. Ivey, USPTO 35552, certifies the filing of this document by facsimile transmission 10/3/03.

Application No. 09/804,613

regarding Anthocyanin, which is a red pigment, has an absorption band spanning the 500-550 nm region, which improves classification or predictive performance, particularly for firmness,

The spectrometers 170 used in the preferred embodiment have charge-coupled device (CCD) array detectors 200 with 2048 pixels or channels, but other array detectors 200, other light detectors 80, including other detector 200 sizes vis-a-vis array size or other method of detector size characterization, may be used as would be recognized by one of ordinary skill in the art. One of the two spectrometers 170 monitors the light source 120 intensity and wavelength output directly, providing a light source reference signal 81 that corrects for ambient light and lamp, detector, and electronics drift which are largely caused by temperature changes and lamp aging. The other spectrometer(s) 170 receives the light detector 80 signal output 82 from one or more light detectors 80 which are sensing light output from one or more samples 30 and/or one or more locations on a sample 30, e.g., at multiple points over a single sample 30, such as an apple, or at multiple points over a sample conveyor 295 belt of apples, grapes or cherries, or a different sample 30, e.g., a different lane on a packing/sorting line, can be measured with each additional spectrometer 170. Each light sensor, e.g., light detector 80(photodetector 255 or other light sensing apparatus or method), in the preferred embodiment represents a separate sample 30 or different location on the same sample 30 or group of samples 30. Spectra from all spectrometers 170 are acquired, in the preferred embodiment, simultaneously. Depending on the type of spectrometer, A/D conversion can occur in parallel or series for each spectrometer (parallel preferred). The computer then processes the spectra and produces an output. Current single CPU computers process spectra in series. A dual CPU computer, two computers, or digital signal processing (DSP) hardware can perform spectral processing and provide output in parallel.

In an alternative embodiment spectra from the wavelength region from about 250-1150 nm, the near-infrared spectra, is examined from samples 30, e.g., fruit including apples. In this particular experiment, a reflectance fiber-optic probe was

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used as the light detector 80. While the spectrophotometer 170 used to collect the data, i.e., sense the spectrum output 82 from the light detector 80, was a DSquared Development, LaGrande, Ore., Model DPA 20, one of ordinary skill in the art will recognize that other spectrometers and spectrophotometers 170 may be used. The spectrophotometer 170 referenced employed a five watt tungsten halogen light source 120, a fiber-optics light sensor to detect the spectrum or output 82 from the sample 30 and provide the light sensor signal input 82 to the spectrometer 170. Other lamps 123 or light sources 120 may be substituted as well as other light sensors or light detectors 80. The light detector signal input 82 to the spectrometer 170, in this embodiment, is detected by a charge coupled device array detector 200. The output from the charge coupled device array detector is processed as described above. Firmness and Brix were measured using the standard destructive procedures of Magness-Taylor firmness ("punch test") and refractometry, respectively. In this embodiment the NIR spectra is detected by an array detector 200 which permits recording or detection of 1024 data points. The 1024 data points are smoothed using a nine-point gaussian smooth, followed by a 2nd-derivative transformation using a "gap" size of nine points. Partial least squares (PLS) regression was used to relate the 2nd-derivative NIR spectra to Brix and firmness. To ensure that false correlation was not occurring, the method of leave-one-out cross-validation was used to generate standard errors of prediction. In cross-validation, the prediction model is constructed using all but one sample; the Brix and firmness of the sample left out is then predicted and the process repeated until all samples have been predicted. The validated model can then be used to nondestructively predict Brix and firmness in unknown whole fruit samples. This information guides harvest decisions indicating time to harvest, which fruit is suitable for cold storage, where the fruit is classified from acceptable to unacceptable characteristics of quality or consumer taste, which fruit to be removed from the sorting/packing operation as not meeting required characteristics, e.g., firmness, Brix, color and other characteristics.

This disclosure of embodiments of an apparatus and method is directed to the simultaneous measurement and use of more than one spectral region from a sample. In this embodiment the use of the chlorophyll absorption region and the NIR region, including the highly absorbing 950-1150 O-H region, is accomplished by exposing the sample, e.g. apple, to more than one intensity source of light or by exposing the light detector 80 at more than one exposure time, e.g., a dual intensity source of light or at least two intensities of light, or by detecting light from a sample with more than one light detector 80 such that each light detector 80 is sensitive to a different spectrum, e.g., by filtering one or more light detectors 80 with filtering either between the sample 30 and the light detector 80 or between the light detector 80 output 82 and the spectrometer 170 input. Fig. 1 illustrates filtered light sources 120 allowing exposure of the sample 30 to different light intensities. Fig. 2 illustrated the use of more than one light detector 80 where filtering between the sample 30 and light detector 80 allows detection of different spectral regions. Shown in Fig. 3A, where the light source is a plurality of discrete wavelength LEDs 257, is an embodiment wherein the sample is exposed to a plurality of light intensities. The intensity of the light source 120 will be selected to provide light output to the light detector 80 which will give optimal S/N data in the desired spectral region. In a first pass a light source, e.g., a lower intensity light source, is used to illuminate the sample, e.g. apple, to obtain data, with an acceptable S/N ratio, in the 700-925nm region. At higher (>925 nm) and lower (<700 nm) wavelengths, the spectrum is dominated by noise due to the low light levels and is not useful. In a second pass a higher intensity light source is selected to illuminate the sample, saturating the detector array at the 700-925nm regions while obtaining data with an acceptable S/N ratio, in the red pigment region of 500-600 nm, the chlorophyll region of 600-699nm and in the O-H region of 926-1000nm. The data from each of the two passes comprises separate data inputs delivered to an analog to digital converter for computer processing. Same spectrometer and A/D for benchtop unit, where the two spectra are acquired sequentially. For on-line, two spectrometers are used, each with its own A/D. In one

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embodiment A/D cards external to the computer are utilized which are serial and are provided by Ocean Optics. This process is provides for multiple channels into a data analyzer for analysis by software. In this embodiment Ocean Optics drivers, hereafter referred to as drivers, accept MS "C" or Visual Basic to 1) determine the spectrum detected from the sample or 2) subject the data to the predictive algorithm and produce the output. Display control computer programs or software periodically requests drivers to deliver the spectrums to be combined. The digital combination then produces, with standard display software, the output display representing the entire spectrum ranges detected from the each sample. There may be, for each sample, multiple spectrum data. For example the spectrum sampling protocol may seek 50 spectrum samples during each of the multiple passes, e.g., 50 spectrum samples during the pass subjecting the fruit sample to the lower intensity light source and separately 50 spectrum samples during the pass subjecting the fruit sample to the higher intensity light source. The total duration of each pass will be determined by the speed of the sorting/packing line and may be limited to approximately 5ms per sample. However, it will be recognized, for all embodiments and sample types, that other sampling times and strategies will be within the realm of use for the invention disclosed herein as different samples and different embodiments are employed. Where the samples being processed, on a sorting/packing line, are apples, there is expected to be little space between each successive apple. Spectrum obtained from the space between apples and at the leading and trailing sides of the sample or apple will be discarded. As the sample, i.e., apple or other fruit, moves under the light detector 80, the spectrum data detected will be that exiting the sample 30 representative of the portion of the sample 30 constituting the path between the point of exposure of the sample 30 with the light source 120 and the point of spectrum exit for detection by the light detector 80. By mathematical inspection of each spectrum, e.g., automated inspection via a computer, this method can determine whether light detected by the light detector 80 is from an apple or the empty space between apples in a sorting/packing line sample conveyor 295. This method can also detect the

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leading and trailing edges of an apple as it passes by the light detector 80 having an output 82 to a spectrometer 170. From this data, discrimination can occur to select specific spectra samples which, for example, are expected to be from the midsection of the sample or apple. Using mathematical inspection of each spectrum (on-line) to determine if it is a good apple spectrum or a spectrum of the line material. The cycle detected by the light detector 80 thus, for each sample 30 in the on the sample conveyor 295 of a sorting/packing line, is composed of an initial segment where the light detector 80 or pickup fiber is exposed to only ambient light with a light shield 284 between the light detector 80 and the light source 120. As the sample 30, e.g., apple, moves into contact with and under the light shield 284, which may for example be a curtain 285, the leading edge or side of the apple will commence to be revealed permitting the light detector 80 to detect spectrum output 82 from the apple. Continued movement of the sample 30 under the light shield 284 exposes the light detector 80 to spectrum output 82 from the sample 30 until the sample 30 moves to the point where the trailing edge or side of the sample 30 is remaining exposed to the light source 120. The sample 30 then moves past the light shield 284 and all light from the light source 120 is blocked between the light detector 80 and the light source 120. Thus the initial spectra detected by the light detector 80 will be at the leading edge or side of the sample 30 as it approaches the curtain 285. The intermediate spectrum measurements, between the initial time at which the leading edge of the sample 30 is exposed to the light source 120 and the time when the trailing edge or side of the sample 30 is exposed to the light source 120, will include those where the light detector 80 or light pickup is optimally positioned to detect spectra most representative of the characteristics of the light spectra output 82 from the sample 30 as the light source 120 illuminates the sample 30, e.g., apple, other fruit or other O-H, C-H or N-H materials. In the preferred embodiment, for ease of data processing, the light detector 80 analog output 82 is converted to digital data by an A/D card. Computer program or software tests the data for acceptance or discarding. The criteria for acceptance of each spectrum sample 30 is a predetermined spectral feature

determined by the expected spectral output 82 of the sample 30, e.g., where the sample 30 is an apple, i.e., the criteria will be to detect a spectrum from 250 to 1150nm falling within the spectra expected for an apple. The detection of the space between apples, in the sorting/packing line, will be recognized as not apples. This spectrum acquired for each sample 30 is the input to the predictive algorithms as indicated by the flow diagram of Fig. 1C. Multiple spectrum, for example fifty spectrum, are detected by the light detector 80 for each sample. The computer program compares each detected discrete spectrum with an expected spectrum from the particular sample, the spectrum not meeting the criteria are discarded, the retained spectrum, e.g., 40 - 50 samples, are combined to provide the spectrum which becomes the input for the predictive algorithm. Multiple spectra from the same apple are averaged to provide a single average spectrum representing multiple points on the apple. the apple may be spinning as it travels by the sensor, e.g., clockwise or counter clockwise in relation to the direction of sorting line travel with better measurement indicated with counterclockwise motion of the sample, thus giving even greater coverage of its surface. Once the average absorbance spectrum for a sample is calculated, the spectrum is multiplied by the regression vector (via a vector multiplication dot product). The regression vector is obtained from previous calibration efforts and is stored on the computer. There is a separate regression vector for each parameter being predicted - e.g., firmness, Brix. The results of the processing the spectrum output 82 by the predictive algorithms will determine the predicted characteristics of the sample 30. The characteristics determined for each discrete sample 30, e.g., apple or other fruit, will be used for decision making in handling or disposition of the sample 30 including, for example, 1) in the packing/sorting line different characteristics will be used for sorting and packing decisions, e.g., by color, size, firmness, taste as predicted by acidity and Brix and 2) characteristics indicating spoilage may trigger methods of elimination of the particular sample 30 from the packing/sorting line.

Packing and sorting of apples will likely involve multiple packing/sorting illumination or light source 120 and light detector 80s for each line. Where the sample 30 is comprised of smaller fruit, e.g., cherries or grapes, there may be multiple light sensors with single or multiple light to interrogate or examine and gather data from a tray of such smaller fruit rather than on the basis of examination of each discrete cherry or grape. For each sample 30, data is acquired, tested to determine if the data corresponds to preset criteria with data selected which meets preset criteria and discarded if it fails to meet preset criteria. Data received by light sensors is then combined to compose the total spectrum sampled. The total spectrum is then compared with the predictive algorithm and decisions are made regarding the sample 30 including, for example, sorting/packing decisions. The results of the comparison of the total spectrum with the predictive algorithm provides a number or other output for end use including information for computer directed sorting equipment.

Operation of the light source 120 is enables the rapid acquisition of reproducible data with good S/N, even in the highly light scattering and absorbing 250-699 nm and the strongly absorbing >950 nm region. The lamp 123 in the preferred embodiment is a 12-Volt, 75-Watt tungsten halogen lamp. However, other light sources which may be used include but are not limited to light emitting diode, laser diode, tunable diode laser, flash lamp and other such sources which will provide equivalent light source and will be familiar to those practiced in the art. The lamp is held at a resting voltage of 2-Volts. When a measurement is taken, the lamp is ramped up to the desired voltage, a brief delay allows the lamp output 82 to stabilize, then spectra are acquired. After data acquisition, the lamp is ramped down to the resting voltage. This procedure extends lamp life and prevents burning the sample. In high speed operations the lamp may always be lighted, e.g., on a high-speed packing/sorting line or used on harvest equipment, and a light "chopper" or shutter or other equivalent article or method could be utilized to deliver light to the passing sample for a determined period of time. The operation of the light source is

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important in extending lamp life, reducing operating expense and reducing disruption of operations. The lamp 123 voltage is ramped up and down to preserve lamp 123 life and to lessen the likelihood of burning fruit. A standby voltage to keeps the lamp 123 filament warm. An ambient/room light background measurement is made to correct for the dark spectrum, which may include ambient light. It is stored and subtracted from the sample and reference (if applicable) so that there is no contribution of ambient light to the sample spectrum, which would affect accuracy.. Dual intensity illumination is employed to: 1) improve data accuracy above 925 nm and below 700 nm and 2) to normalize path length changes due to scattering. Dual exposure time increases the likelihood of increased data quality with large and small fruit. Utilization of more than one light detector 80, with each positioned at different distances from the sample, will likewise increase the ability to obtain increased data quality throughout each portion of the spectrum from approximately 250nm to 1150 nm.

Other steps in determining predictive algorithms included reference determination of pH using electrode measurement and reference determination of total acidity using end-point titration of extracted juice. Correlation between the NIR spectra and the reference data (pH and total acidity) was conducted. Methods known to those practiced in the art such as partial least squares (PLS) are used to determine the correlation of the NIR spectrum with a chosen parameter such as pH.. Once correlation is established, PLS is used to generate a regression vector from the calibration samples. This regression vector is then used to predict sample properties by taking the dot product of the sample spectrum and regression vector. NIR analysis can be carried our directly on the juice yielding very high correlations with Brix, pH, and total acidity. A commercially available "dip probe" is used that is a common item available from optical fiber fabricators or from companies involved in process analysis. In addition to the use of PLS for quantifying Brix, firmness, pH and acidity, Principal Components Analysis (PCA) was performed on the NIR spectral data. PCA differs from PLS in that no reference data is required. PCA allows classification of

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firm vs. soft apples and low pH vs. high pH samples. This classification algorithm is sufficient to achieve the goal of product segregation. Using PCA, poor quality fruit can be removed from a batch and the highest quality fruit can be segregated into a premium class. Poor quality fruit was observed to often have a higher pH level than good quality fruit.

Fig. 4 illustrates an alternative embodiment of the disclosure and includes at least one light source 120 transmitted by a transmitting article, for example a fiber optic fiber or other equivalent article for transmitting light; a sample 30 having an sample surface 35; input mechanism of positioning light from the at least one light source 120 proximal the sample surface; at least one illumination detector; output mechanism of positioning the at least one illumination detector proximal the sample surface; the at least one light source 120 and the at least one illumination detector may be positioned in relation to the surface or against the surface by a positioning article provided, for example, by a positioning article spring biased against the surface of the sample; the pressure against a sample surface, by an at least one light source 120 or an at least one illumination detector, will be limited by surface characteristics of the sample and/or the character of the measurement process, i.e., pressure may be reduced where a sample is subject to surface damage or where the measurement process is in at high speed limiting the time permitted for each separate sample contact. The illumination is transmitted to the surface, for example by fiber optics or other equivalent manner; and at least one device or method of measuring the illumination detected from the sample. The light source, for the disclosure herein may be a lamp, which for example, but without limitation, may be a tungsten halogen lamp or the equivalent, which may produce a spectrum within the range 250-1150 nm and have a filament temperature of of 2500 to 3500 degrees kelvin; other broadband spectrum lamps may be employed depending upon the sample 30, characteristics to be predicted, and embodiment utilized; the at least one device or method of measuring the illumination may be a spectrometer having at least one input; the at least one spectrometer may include, for example, a 1024 linear array detector with

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those of ordinary skill in the art recognizing that other such detectors will provide equivalent detection; the at least one illumination detector may be a light pickup fiber or other equivalent detector including for example a fiber optics light pickup; the at least one illumination detector collects a spectrum which is received by the at least one spectrometer input; the sample in this embodiment is from the chemical group of CH, NH, OH or the physical characteristics of firmness, density, color and internal and external defects. Additionally, the light source 120 may comprises a plurality of illumination fibers. In this embodiment a plurality of illumination fibers may be arrayed such that each of the plurality of illumination fibers is equidistant from adjacent illumination fibers; the at least one illumination detector may, in this embodiment, be positioned centrally in the array of illumination fibers. In an embodiment of this disclosure, the plurality of illumination fibers may, for example, be comprised of 32 illumination fibers and the light source 120 may be provided, for example, by a 5w tungsten halogen lamp or other equivalent light source or by a plurality of illumination sources provided for example by at least two light sources such as, for example, at least two 50 Watt light sources. Illumination sources may be composed, for example, of sources having a focusing ellipsoidal reflector with cooling fan. In this embodiment the at least one illumination detector may comprise a plurality of light detectors 80, which may for example, be arrayed such that each illumination detector is equidistant from adjoining light detectors 80; where at least two light sources are positioned are employed, they may for example be positioned 45 degrees relative to the illumination detectors. in the array of illumination fibers. In an additional embodiment of this disclosure, a plurality of light detectors 80 may be comprised of twenty-two illumination detectors. An embodiment of the disclosure may be comprised of at least one light source 120 composed of a 5 w tungsten halogen lamp; the at least one illumination detector is a single detection fiber; the light source 120 is positioned against the sample 30 degrees distal to the detection fiber. If the measurement of the sample surface is made in a non-contacting manner, an alternative embodiment may include a polarization filter between the light source

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120 and the sample, provided, for example by a linear polarization filter or an equivalent as understood by one of ordinary skill in the art; a matching polarization filter is positioned between the at least one illumination detector and the sample, which may be provided, for example by a linear polarization filter rotated 90 degrees in relation to the polarization filter between the light source 120 and the sample.

The method described above, which uses wavelengths of both visible radiation (250-699 nm) specifically chosen to include the absorption band for yellow color pigments (250-499nm), red color pigments (500-600 nm) and green pigments or chlorophyll (601-699 nm), as well as NIR (700-1150 nm) radiation to correlate with Brix, firmness, pH, acidity, density, color and internal and external defects can be carried out using a variety of apparatuses.

ADDITIONAL DETAILED DESCRIPTION

Overview of calibration of visible/NIR sensors:

Required calibration was addressed in the Parent Application 09/524,329, in paragraphs, identified by page/line by pn/ln, as follows: 1/18; 3/17, 22, 28; 4/2; 8/8; 9/4; 9/14; 12/16; 16/8; 22/5; 31/21; 33/19; 39/10; 43/4; 47/1; 52/13 etc. Calibration of spectroscopic maturity and quality sensors involves building algorithms that relate the visible and near infrared spectrum of an individual fruit or vegetable to one or more of the following: Brix (including, but not limited to sugar content, or sweetness, or soluble solids content); acidity (including but not limited to total acidity, or sourness, or malic acid content or citric acid content or tartaric acid content); pH; firmness (including but not limited to crispness or hardness); internal disorders or defects including but not limited to watercore, browning, core rot, insect infestation. Furthermore, the individual property data collected above can be combined as follows: using the ratio of the sugar content to acid content to better predict eating quality, taste, sweet/sour ratio; using the combined data from two or more of the following: sugar content, acid content, pH, firmness, color, external and internal disorders to better predict eating quality.

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<u>Integrating visible/NIR sensors with packing, sorting and conveyance systems and synchronizing data acquisition with product location/position to optimize collection of sample data, and reference and standardization data.</u>

Sensing sample data including the presence or absence of a sample was addressed in the parent in paragraphs, identified by page/line by pn/ln, as follows: 20/20; 36/8 etc. Using spectroscopic sensors for measuring fruits and vegetables while in motion on a sample conveyor 295 system in sorting and packing warehouses is illustrated in Fig. 10 and Fig. 10A and is done as follows: The presence or absence of a sample 30 and the position/location of the sample 30 relative to the point of spectrum measurement is determined using one or more of the following means: 1) sample 30 position determination means and or sample conveyor 295 position determination means, provided for example by an encoder or pulse generator 330, as seen in Fig. 9, integral to the sample conveyor 295 and detecting sample conveyor 295 movement, provides one or more electronic or digital signals to a CPU 172 which initiates, by computer program control, control signals to initiate and stop acquisition of spectra, 2) the spectrum itself is automatically inspected using computer programs or programmed hardware, e.g., digital signal processors, to determine if the sample 30 being measured is at the optimal location(s) for spectrum measurement, 3) a proximity sensing means 340, including proximity sensors of, but not limited to, magnetic, inductance, optical, mechanical sensors; and also known as object presence sensors, such as thru-beam or reflectance sensors 341, is used to provide information about the position, i.e., orientation or location of the product on the packing or sorting line relative to the NIR sensor, e.g., light detector 80, and/or size of the sample 30, such proximity sensing means 340 and their use being of common knowledge to those practiced in the art of industrial processing object presence sensing. The proximity sensing means 340 can be placed 1, 2, 3 or ...n units of length, e.g., cups or pockets or conveyor belt length, before the NIR sensor, e.g., detector 80, to indicate if 1, 2, or 3 or...n more empty spaces, e.g., cups or pockets or a defined and known length of conveyor belt, are present in sequence, thus

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allowing a greater amount of time for performing dark spectra and/or reference spectra and/or standard/calibration samples. Using one or more of the above methods, the presence or absence of sample(s) 30 is determined over a defined length of the particular sample conveyor 295 system. If sample(s) 30 is present, multiple visible and near-infrared spectra are acquired as the sample 30 passes by the light source 120 lamp(s) 123 providing light detector output 82 and spectrometer(s) 170 detector 200 input; such light collection may be achieved using a collimating lens 78 and or other light transmission means including for example fiber-optics to transfer the light that has interacted with the sample 30 to the spectrometer(s) 170 detectors 200. If no sample 30 is present, other reference measurements are made to improve stability and accuracy such as previously mentioned dark spectra, reference spectra (lamp intensity and color output), and standard/calibration samples, which may be optical filters or polymers or organic material with known and repeatable spectral characteristics. Measurements that are made when no sample is present include, but are not limited to 1) measuring a reference spectrum (intensity vs. wavelength) of the light source(s), 2) measuring the dark current (no light conditions) of one or more spectrometer(s) 170 detector(s) 200, including but not limited to the sample spectrometer(s) 170 and the reference spectrometer(s) 170, and 3) standard or calibration samples or filters 130 or material.

Obtaining a spectrum of the lamp(s) for determining reference light output and obtaining baseline dark current spectra from detector(s). Both reference and dark spectra are used with sample spectrum to calculate the product's absorbance spectrum.

Reference to reference, baseline and dark spectra was addressed in the parent in paragraphs, identified by page/line by pn/ln, as follows: 12/18; 39/10; 52/14 etc. The reference measurements to account for changes in light source intensity or color output can be obtained using a reference light transmission means 320, e.g., a fiber-optic bundle which may be furcated, a light pipe or other means of transmitting light, with a common end 322 providing input to a reference spectrometer 170, and, where

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furcated, one or more branched ends 81, each of which is mounted by means to allow only light from the light source 120 lamp(s) 123 to enter the reference light transmission means 320. A light shutter 300 is placed between each light source 120 lamp 123 and each reference light transmission means 320. The at least one light shutter 300 can be opened and closed separately by shutter control means 305 including, for example, driven by a linear actuator or rotary solenoid or other mechanical or pneumatic device, or all at once.

Each light source 120 lamp 123 in the system can be measured separately to determine if it is faulty or if it will soon need replacement based on a stored intensity vs. wavelength spectrum profile. The combined intensities from the reference light transmission means 320 is used as the reference spectrum for purposes of calculating an absorbance (or log 1/R) spectrum, which is linear with concentration (e.g., percent Brix or acidity or pounds of firmness, etc.).

Closing all of the light shutters 330 of the reference light transmission means 320 allow a dark current (no light condition) measurement of the spectrometer 170 detector(s) 200. The dark current is largely affected by temperature and must be periodically measured and its intensity value at each wavelength (or detector) pixel subtracted from the reference spectrum obtained with the shutters 330 open.

The sample spectrometer's 170 detector 200 dark current must also be periodically measured by closing light shutters 330 that are placed between the light source and the sample 30, or between the sample 30 and the sample spectrometer light collection fiber, seen here as detector 80 and detector output 82, or between the light collection fiber and the spectrometer 170. Similarly to the reference measurement, the dark current of the sample spectrometer 170 must be subtracted from the sample spectrum obtained with the shutters 330 open. It will be appreciated that reference measurement must be made with respect to the spectrometer 170 used for light source 120 lamp 123 measurement as well as for the spectrometers 170 used to acquire detector 80 spectrum output 82 as processed in the computer program

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controlled CPU 172 in association with algorithms for the characterization of samples 30.

The reference measurement, utilizing a shutter means, is demonstrated in Fig. 9. Fig. 9 is an elevation depicting an additional embodiment of the invention demonstrating at least one light detector 80 having at least one output 82 to at least one spectrometer 170 having at least one detector 200. At least one colluminating lens 78 intermediate the at least one light detector 80 and a sample 30. The at least one light detector 80 positioned to detect light from the sample 30. At least one light source 120 lamp 123; a shielding means intermediate the at least one light source 120 lamp 123 and a sample 30 conveyed by sample conveyor 295. At least one aperture 310 in the shielding means to allow illumination of the sample 30 by the at least one light source 120 lamp 123. It will be appreciated by those of ordinary skill in the instrument containment arts that an instrument case or container will be a means of mounting the elements of the disclosed invention in all its embodiments. It will be appreciated that a case 250 may provide shielding and mounting means for the invention. At least one light interruption means intermediate the at least one light source 120 lamp 123 and the at least one aperture 310. Light interruption means provided, for example, by light shutter 300 means. The at least one light shutter 300 operable by at least one shutter control means 305, e.g., linear actuator or rotary solenoid operated by means, e.g., mechanical driven by electrical, pneumatic, hydraulic or other power means or other shutter means including for example liquid crystal screen operated by means. The at least one shutter control means 305 receiving control signals from at least one CPU 172 having at least one shutter operating control output 307. At least one reference light transmitting means 81 including, for example, fiber-optics including bifurcated fiber-optics, receiving reference light output from the at least one light source 120 lamp 123. At least one reference light interruption means, comprised for example of shutter 301, intermediate the at least one light source 120 lamp 123 and the at least one reference light transmitting means 81. The at least one reference light shutter 301 operable by

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at least one shutter control means 305, e.g., linear actuator or rotary solenoid operated by means, e.g., mechanical driven by electrical, pneumatic, hydraulic or other power means or other shutter means including for example liquid crystal screen operated by means. The at least one reference light shutter 301 shutter control means 305 receiving control signals from at least one CPU 172 having at least one shutter operating control output 307. The at least one reference light transmitting means 81 providing an input to the at least one spectrometer 170 detector 200. The at least one CPU 172 providing at least one lamp power output 125 to the at least one light source 120 lamp 123. The at least one spectrometer 170, receiving input from at least one reference light transmitting means 81 having at least one output 82 received as in input to the at least one CPU 172. The spectrometer output 82 capable of A/D conversion to form input to the at least one CPU 172. The at least one spectrometer 170, receiving input from at least one detector output 82 received as in input to the at least one CPU 172. The spectrometer output 82 capable of A/D conversion to form input to the at least one CPU 172. Mounting means to light sources 120 lamps 123, detectors 80, shutters 300, shutter control means 305, reference light transmitting means 81 and case 250. Encoder/pulse generator 330 input to CPU 172 providing sample conveyor 295 movement data. Computer program to operate CPU 172 in data collection and control functions.

A reference measurement of the light source 120 lamp(s) 123 intensity vs. wavelength output can also be obtained using reflecting means 360, as seen in Fig. 11, including but not limited to, for example, mirrors or other reflecting or diffusing material, including roughened aluminum, gold, Spectralon®, Teflon, ground glass, steel. Reflecting means 360 will be positioned to reflect light source 120 lamp 123 light to a detector 80 having an output 82 received by a spectrometer 170 detector 200. A colluminating lens 78 may be positioned intermediate the detector 80 and the light reflected by the reflecting means 360. Reflecting means 360 may be positioned, e.g., inserted via an aperture 310, for example where a case 250 is utilized, when a reference measurement is to be made as dictated by reflecting control means 308 as

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an output from a CPU 172. The CPU 172, via means, will detect the presence or absence of a sample 30 and, when a sample 30 is absent for "n" time increments or sample conveyor 295 movements will provide a reflecting control means 308 control signal to reflecting position means 306, e.g., linear actuator or rotary solenoid operated by means, e.g., mechanical driven by electrical, pneumatic, hydraulic or other power means. The reflecting means 360 capable of being withdrawn as dictated by reflecting control means 308 as an output from the CPU 172 when reference measurement is to be ceased and spectra measurement of a sample 30 resumed.

A light reflecting or diffusing body for obtaining the reference spectrum may also be obtained by mechanical insertion of reference means 430, as seen in Fig. 12 and Fig. 13, in or near the location where actual sample 30 is normally measured, which is between the light source 120 lamp(s) 123 and reference light transmission means 320 leading to the sample spectrometer 170 detector 200(s). Insertion is by insertion means including but not limited to an actuator system 400 capable, upon receiving control signals or means as recognized by those of ordinary skill including control signals or means provided from a CPU 172, of operation of an actuator 410 causing a piston 420 to extend 421 and retract 422 as seen in Fig. 12 and 13. Power, including for example electrical, pneumatic, hydraulic and other means, is provided to operate the actuator by power transmission means 440 as will be appreciated by those of ordinary skill.

A CPU 172, controlled by computer program, is not depicted in Fig. 10, 10A, 11, 12 or 13 as a person of ordinary skill will appreciate such structure from viewing other drawings presented herein.

Achieving whole product measurement (minimizing errors due to localized measurement).

To improve the measurement of the entire product, two or more light sources 120 lamps 123 and/or detection 80 points are used. The product can be measured rolling or not rolling with a rolling measurement generally improving whole product

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measurement, while a non-rolling measurement provides better accuracy and introduces less spectral noise due to movement.

As a single fruit or vegetable sample 30 passes by the point of spectrum acquisition, multiple spectra are acquired, each spectrum representing a different measurement location or area on the product.

<u>Optimizing signal-to-noise and accuracy with small and large size product.</u>

One or more means may be used to determine the size or weight of the individual fruit or vegetable sample 30. Means for determining product size includes, but is not limited to 1) a separately determined weight or mass using sensors common to the industry, 2) utilizing the color sorter or defect sorter data (e.g., from camera or CCD images), 3) utilizing other size sensors based on magnetic, inductive, light reflectance or multiple light beam curtains, common to other industries. The relative size of the sample 30 can then be used to adjust the hardware spectrum acquisition parameters or the amount of light (by varying the aperture 310 size) to provide an improved signal-to-noise ratio spectrum for large samples 30 and/or to prevent detector 80 saturation by light for small product sample 30, e.g., detector 80 exposure or integration time can be set for longer time periods for large product samples 30 and for shorter time periods for small product.

<u>Improving accuracy by inspection of multiple individual spectra collected from a single product and removing poor quality or "outlier" spectra. Then, calculating the absorbance spectrum from the raw data collected for dark, reference and sample.</u>

Each individual spectrum from the series of spectra acquired for each individual product sample 30 are then inspected by a computer program or programmed hardware. Poor quality spectra are deleted from this batch of spectra and the remaining spectra are used for constituent or property prediction. The retained spectra of the product are combined with the appropriate reference and dark current measurements to produce an absorbance spectrum as follows:

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Absorbance Spectrum = -log10 [(sample intensity spectrum- sample dark current spectrum) / (reference intensity spectrum – reference dark current spectrum)] i.e. the absorbance spectrum is equal to the negative logarithm (base 10) of the ratio of the dark current corrected sample spectrum to the dark current corrected reference spectrum.

All of the absorbance spectra for each product sample 30 can then be combined to produce a mean or average absorbance spectrum of the product sample. This average absorbance spectra can then be used to compute the component or property of interest based on a previously stored calibration algorithm. Alternatively, each absorbance spectrum can be used individually with a previously stored calibration algorithm to compute multiple results of the component or property of interest for an individual product, followed by determination of the average or mean component or property value computed by summing all of the values and dividing the resultant sum by the number of absorbance spectra used.

Method for measuring samples and importance of linking location on product where visible/NIR data was collected with the same location that will be measured by the laboratory reference technique.

Calibration is performed as follows: 1) Spectra of product sample 30 are measured and absorbance spectra (corrected for reference and dark current) are stored, 2) Standard laboratory measurements (which are often destructive) are made on the product sample 30. Note: it is important to the success of the NIR method that the portion of the sample 30 that is interrogated between the light source(s) 120 lamps 123 and light collection(s) detectors, e.g., light detectors 80, leading to the spectrometer(s) 170 detectors 200 is the same as that portion measured by the standard laboratory technique.

For many sample conveyors 295 that are used for whole fruit and vegetable sorting and packing operations, the product can be transported past the NIR measurement location rolling or not rolling. If absorbance spectra are collected from the product as it is rolling, the exact location of any one measurement (one spectrum)

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is not usually known, and therefore the entire product (as opposed to one localized spot) must be analyzed for the component or property of interest. If calibration algorithms are constructed in this way (using measurements of rolling product), all of the retained spectra for that individual product are averaged to produce an average absorbance spectrum and the total product component or property is assigned to this one absorbance spectrum.

Because most fruits and vegetable are heterogeneous and vary in component level with location, it is preferable to develop a calibration model on product sample 30 that is not rolling so that each acquired spectrum is from a known physical location on the product sample 30. Then, laboratory measurements are made on the same portion of product sample 30 that spectra were taken from. When this procedure is used, a whole fruit or vegetable sample 30 may be separated, e.g., cut or sliced, into smaller sub-portions prior to laboratory analysis. These smaller sub-portions each correspond to NIR data collected over the same locations within the product sample 30; the time period of NIR data acquisition can be adjusted to shorter or longer times, corresponding to the measurement of smaller or larger product samples 30, respectively. In this case, each sub-portion of the product sample 30 will have one or more spectra associated with that particular location. The laboratory determined component or property is then assigned to each spectrum or spectra from that particular location.

Mathematical processing is performed on absorbance spectra prior to conducting statistical correlation analysis and calibration model building.

Absorbance spectra are pre-processed using a bin and smooth function. Partial least squares analysis (or variants thereof such as piecewise direct standardization) are then used to relate the processed absorbance spectrum to the assigned component and property values such as Brix, acidity, pH, firmness, color, internal or external disorder severity and type, and eating quality.

Method to minimize the number of samples needed to develop a calibration model.

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To minimize the number of calibration samples that are necessary, the following method can be used: 1) spectra are collected on all test samples 30, 2) prior to destructive laboratory measurements, principal components analysis (PCA) is performed on the absorbance spectra, 3) Resultant Score plots from PCA (e.g., Score 1 vs. Score 2, Score 3 vs. Score 4, etc.) are then generated, 4) A subset of the original samples (e.g., 40% of the original number of samples) are selected from the Score plots in either a random fashion or by selecting samples that, as a group, yield a similar range, mean and standard deviation of score values compared to the entire group of original samples 30.

Calibration updates are periodically required to maintain measurement accuracy, particularly with agricultural product samples 30 that can vary in composition with growing conditions and variety. Several methods can be used to minimize the efforts of calibration updates. As fruit or vegetable samples 30 are analyzed in a packing and sorting warehouse, their visible/near infrared spectra can be examined by software to determine if the sample qualifies as a potential calibration update sample 30. Good calibration update samples 30 will cover low to high component values and will have Score values that cover the same range as the original sample's 30 Score values.

While a preferred embodiment of the present disclosure has been shown and described, it will be apparent to those skilled in the art that many changes and modifications may be made without departing from the disclosure in its broader aspects. The appended claims are therefore intended to cover all such changes and modifications as fall within the true spirit and scope of the disclosure.

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